STUDIES ON SYNTHESIS, CHARACTERIZATION OF METAL OXIDE NANOPARTICLES AND THEIR MULTI-FUNCTIONAL BIOLOGICAL APPLICATIONS

SYNOPSIS

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BY

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Introduction

In modern material science, nanotechnology stands as the most effective and novel area of research. The rapid developments in this field are posing a valuable impact on various sectors like electronics, environmental science, catalysis, biomedical science, and many others [1].The principles of nanotechnology involved in the design and systematic synthesis of nanomaterials lead to the formation of various nanostructured, core components of nanotechnology viz. nanoparticles (NPs), nanowires, nanorods, nanospheres, nanoclustures, nanoplates, nanofilms, nanodots, and nanotubes [2]. These nanomaterials have been known for hailing tremendous contributions in the big world of nanotechnology. The specialty of these nanomaterials lies in their large surface to volume ratio, quantum effect, and surface defects compared to their bulk counterparts [3]. Among the various nanomaterials, the metal oxide (MO) NPs are emerging as the materials of great interest to the research community; due to their wide range applications in biomedical science, environmental science, electronics, catalysis, and many other fields [4]. Considering the expansive applications of MO NPs, researchers have developed many physical and chemical methods for their synthesis. Although these methods promise the production of MO NPs with desired properties, they tend to exhibit some destructive effects [5].On the other hand, the 'green synthesis' approaches used to fabricate the MO NPs by utilizing the different 'green sources' viz. plants and microorganisms are much trending. The benefit of the green approach over the other traditional approaches is that, the phytochemicals present in the extract of these green sources function as both reducing and capping agents, making the obtained MO NPs more suitable for biomedical and environmental applications [6].

Recently, the synthesis of many pure, doped, and bimetallic MO NPs like zinc oxide (ZnO), copper oxide (CuO), magnesium oxide (MgO), MgZnO, AgZnO, Ag/ZnO, Cu/ZnO, etc., using the green synthesis approaches were reported [7-10]. Among these, the ZnO NPs have been significantly attracting the researchers owing to their distinctive optical and chemical behavior, which could be easily tuned by changing their size and shapes. In addition to this, the ZnO NPs are also known for their photo-catalytic and photo-oxidizing properties of chemical and biological species [11]. These unique catalytic and

oxidizing capabilities make the ZnO NPs as the most suitable candidates for their use in the fields of medicine and environmental science as antimicrobial, anti-inflammatory, anti-tumor, anti-diabetic, biosensing, and as photocatalytic agent for the degradation of organic pollutants responsible for water pollution [12-14].

On the other hand, MgO NPs are important functional MO NPs that have been widely used in the catalysis, refractory material, and in the biomedical field for bone repair, heartburn (as antacid), treating sensitive stomach, and also as anticancer, antimicrobial agent [15]. In addition to this, the doping of these pure NPs with other metals such as silver (Ag), chromium (Cr), and rare earth metals could enhance their catalytic and biological properties by increasing the surface defects [16].

Therefore, considering the far-flung applications of green synthesized MO NPs, some attempts were made to synthesize pure ZnO, MgO NPs and also Zn doped MgO, Ag/ZnO bimetallic NPs using ethanolic and aqueous extracts of plant materials. For the purpose, three medicinal plants viz. *Emilia sonchifolia (L), Sauropus androgynus (L), and Vateria indica (L)* belonging to the family *Compositae, Phyllanthaceae*, and *Dipterocarpaceae* respectively were chosen. The extracts of plant materials taken from the chosen plants were found to be rich in vitamins, polyphenols, carotenoids, tannins, minerals, and antioxidants with excellent medicinal values.

The plant extracts were employed in the synthesis process to play the dual role of reducing and capping agents thus minimizing the use of harmful chemical reductants and stabilizers. The synthesized NPs were well characterized through FT-IR, P-XRD, FE-SEM, EDS, and BET analyses. After confirming the formation of mesoporous NPs with divergent shapes and crystallite size less than 50 nm they were scrutinized for their anti-cancer, antimicrobial, and antidiabetic properties. The studies have revealed a superlative antineoplastic property of synthesized NPs against the tested cell lines [human triple-negative breast cancer (TNBC) cells (MDA-MB468)], an excellent antimicrobial activity against four tested bacteria [*Staphylococcus aureus, Bacillus subtulis, Pseudomonas aeroginosa, Pseudomonas syringae,* and *Escherichia coli*], and appreciable antidiabetic

activity with potential α-amylase inhibition activity. Additionally, the NPs were also evaluated for their haemocompatibility using human erythrocytes.

The detailed desciption on the collection and preparation of plant material extracts, methods, and the process employed in the synthesis of NPs, techniques and the assays used for the material characterization and evaluation of their biological properties and the outcome of the studies along with the literature support have been discussed under the heading "**Studies on synthesis, characterization of metal oxide nanoparticles and their multi-functional biological applications"** in six major chapters as described below.

Chapter 1:

An overview of nanotechnology, nanomaterials, synthesis, and the biological applications of metal oxide nanoparticles

This is an introductory part of the entire research work including a thorough literature review regarding the development of nanotechnology, different types of nanomatrials giving special priority to NPs and their prominence in different fields and the need of green synthesis methods for the production of NPs. This also includes a meticulous literature survey about the production, various properties, and the biological importance of chosen NPs viz. ZnO, MgO, and Ag/ZnO bimetallic, and doped MgO NPs.

Chapter 2a:

Material and Methods

This chapter is a brief description of materials used in the research work along with the introduction to the various characterization techniques including their working principle. It also delineates the various assays used in the evaluation of different biological activities of synthesized NPs.

Chapter 2b:

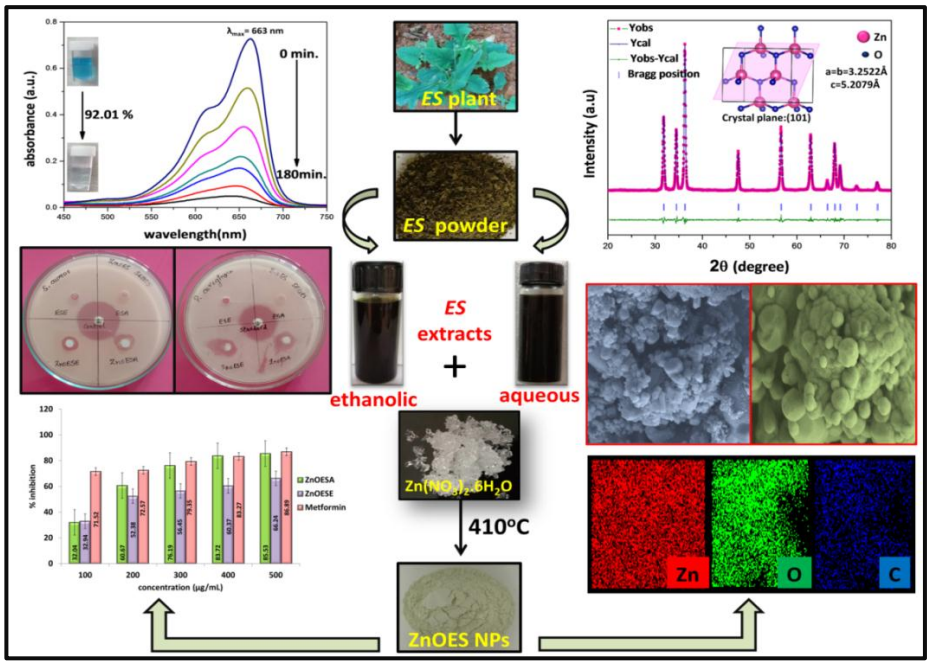
Phytochemical quantification and GC-MS profiling of plant extracts employed in the green synthesis of metal oxide nanoparticles

This chapter gives the information about the scientific classification, parts used, and the medicinal values of chosen plants. This also elucidates the type and quantity of phytochemicals present in their extracts with standardized assays and GC-MS profiling.

Chapter 3:

An ensuing repercussion of solvent alteration on various biological properties of *Emilia sonchifolia* (*L.*) phytochemicals capped biocompatible zinc oxide nanoparticles

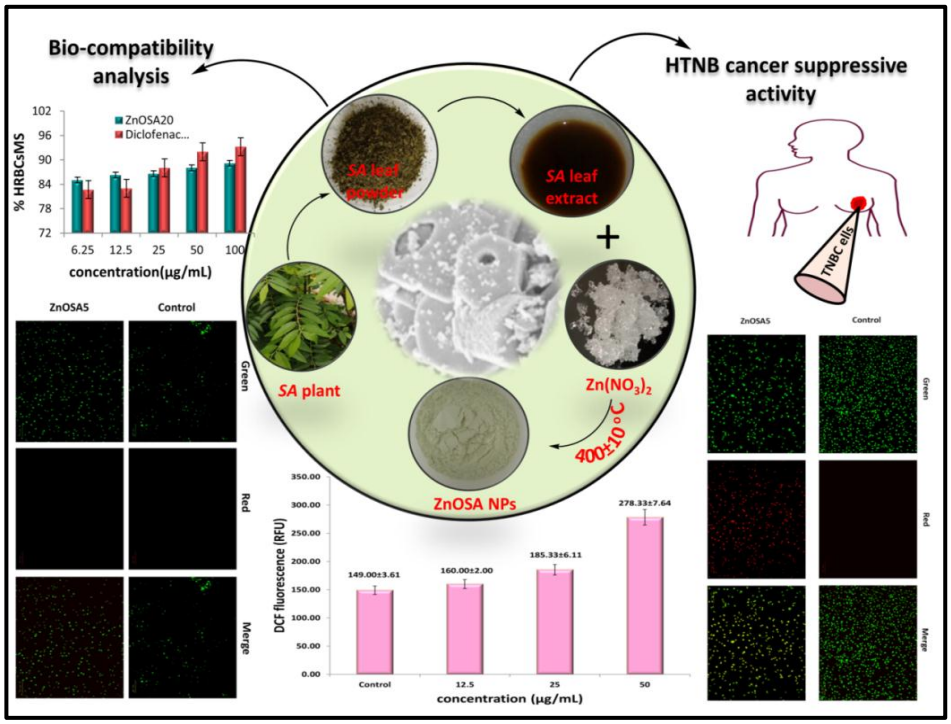
In the framework of this chapter, a triumphant attempt made to obtain biocompatible ZnOES NPs using *E. sonchifolia (L.)* plant extracts has been described. A repercussion of solvent alteration on the biological properties of ZnOES NPs has also been delineated employing two solvents viz. water and ethanol during the extraction of plant material. The formation of ZnOES NPs was confirmed through FT-IR and P-XRD which was further upheld by the elemental mapping study. Additionally, the NPs were examined through Rietveld refinement of P-XRD data obtained, FE-SEM, and BET analyses. The obtained NPs were scrutinized for their *in-vitro* antidiabetic, *in-vitro* anti-inflammatory, and anti-microbial activities. The biocompatibility of ZnOES NPs was accessed via membrane stabilization method using human red blood corpuscles. The results obtained have been discussed in detail.



Chapter 4a:

***Sauropus androgynus* (*L.*) leaf phytochemical activated biocompatible zinc oxide nanoparticles: An antineoplastic agent against human triple negative breast cancer**

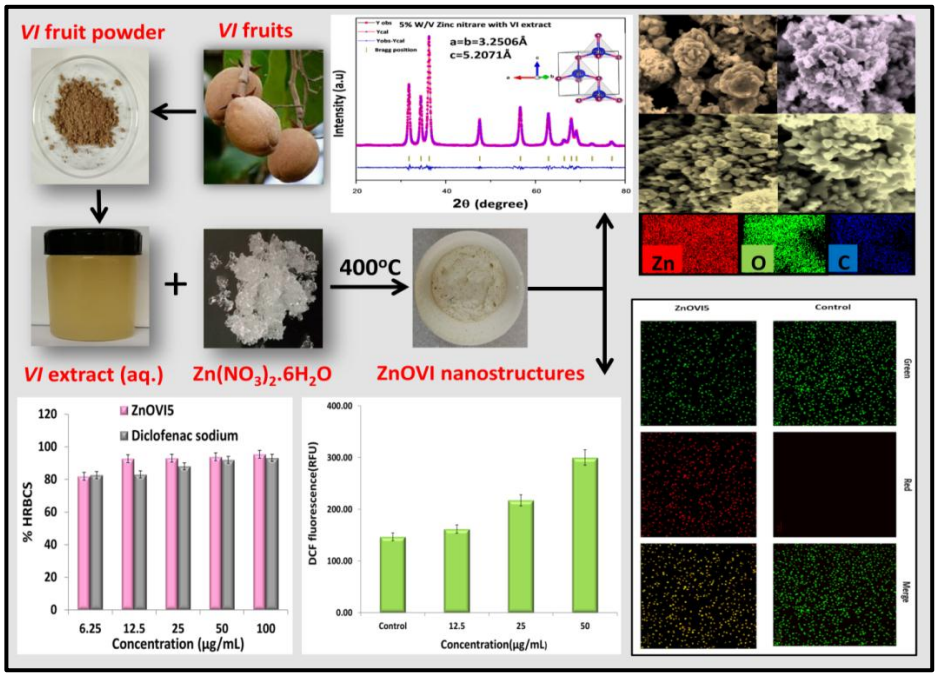
This chapter delineates the biosynthesis of ZnOSA NPs by availing *S. androgynous (L.)* aqueous leaf extract. The formation of ZnOSA NPs was substantiated by FT-IR, P-XRD, FE-SEM, EDS, and BET techniques. The green synthesized NPs were examined for their *in-vitro* cytotoxicity on the most challenging human triple-negative breast cancer (TNBC) cells using MDAMB468 cell line. In addition to this, the biocompatibility of these NPs was tested using NIH3T3 mouse fibroblast cells and human red blood corpuscles. The apoptosis study was performed on TNBC cells using the AO-EB dual staining assay. Furthermore, reactive oxygen species (ROS) measurement study was conducted to substantiate the oxidative-stress induced cytotoxicity. Moreover the NPs were analyzed for their DPPH free radical scavenging potentiality. The details of the study have been discussed.



Chapter 4b:

Unravelling the human triple negative breast cancer suppressive activity of biocompatible zinc oxide nanostructures influenced *by Vateria indica* (*L.*) fruit phytochemicals

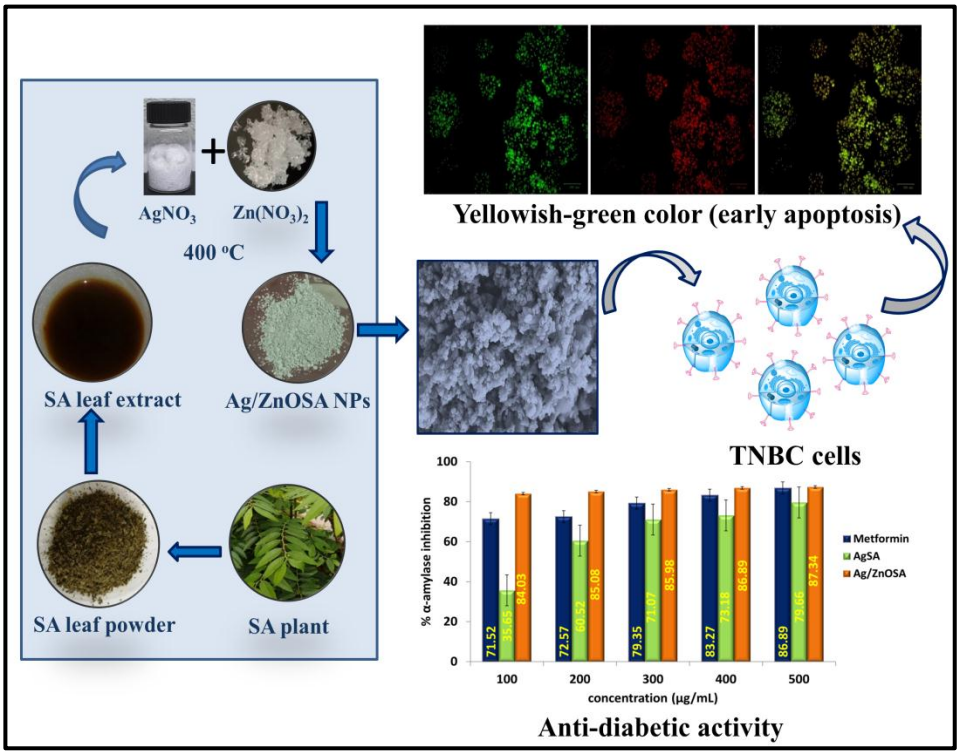
This chapter describes the biosynthesis of ZnOVI nanostructures by using aqueous fruit extract of *V. indica (L.).* The formation of ZnOVI nanostructures was corroborated by FT-IR, P-XRD, FE-SEM, EDS, and BET analysis. The obtained nanostructures were explored for their *in-vitro* cytotoxicity using MDA-MB468 human triple-negative breast cancer (TNBC) cells. The biocompatibility was evaluated by performing MTT assay on the NIH3T3 mouse fibroblast cells and human red blood corpuscles. The AO-EB dual staining assay was followed to study the apoptosis in the TNBC cells. Besides, reactive oxygen species (ROS) measurement study was conducted to bear the witness to the oxidative-stress induced cytotoxicity. Additionally, the nanostructures were studied for their radical scavenging ability using DPPH free radical. The details of the study have been discussed.



Chapter 5a:

Bio-fabrication of Ag and Ag/ZnO nanoparticles using *Sauropus androgynus (L.)* extract and evaluation of their cytotoxicity against MDA-MB468 human triple-negative breast cancer cells

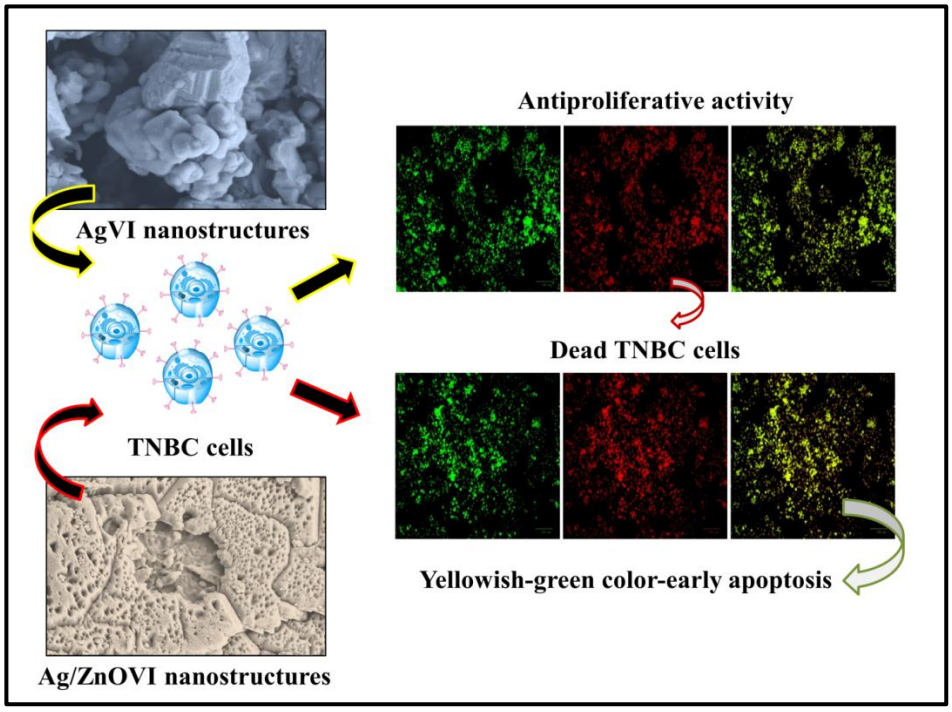
In this chapter the bio-fabrication of pure silver (AgSA) and bimetallic silver/zinc oxide (Ag/ZnO) NPs has been described. The NPs synthesized using *S. androgynus (L.)* extract were analyzed for their crystal phase, size, shape, morphology, and surface properties through FT-IR, P-XRD, FE-SEM, EDS, and BET techniques. Also the *in-vitro* cytotoxic potentiality of these NPs was studied on the most aggressive human triple-negative breast cancer (TNBC) cells. Additionally, the stage of apoptosis was studied via AO-EB dual staining method. Furthermore, the DPPH free radical scavenging capacity, *in-vitro* anti-inflammatory, and *in-vitro* antidiabetic activity was performed. The results obtained have been discussed in detail.



Chapter 5b:

Antiproliferative activity of *Vateria indica (L.)* phytochemical influenced Ag/ZnO nanocomposite against Human Triple-negative breast cancer cells

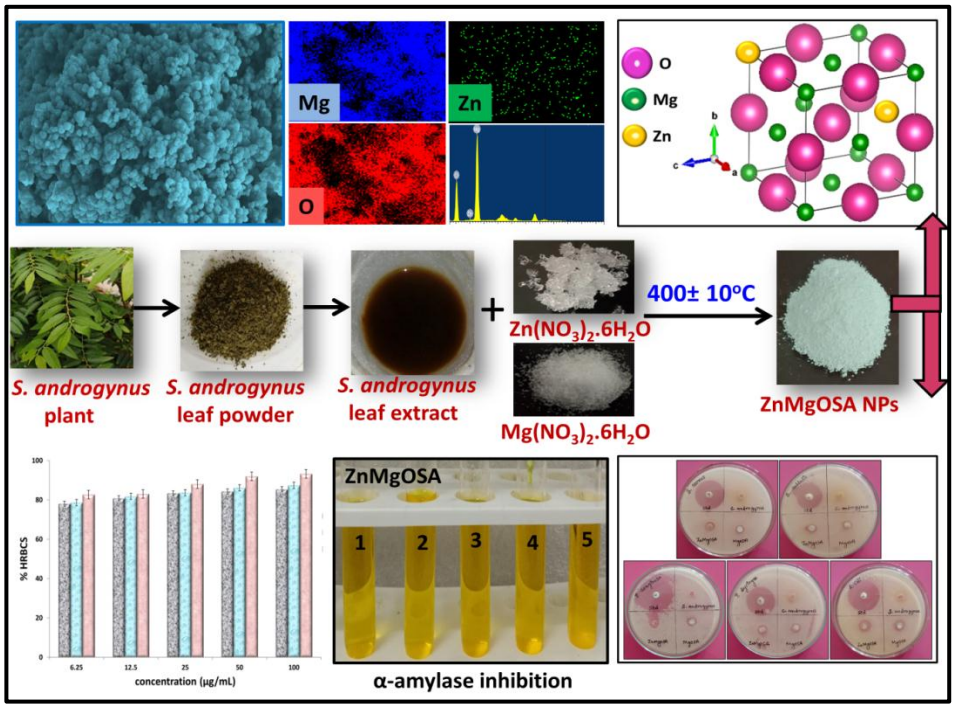
This chapter delineates the synthesis of pure silver (AgVI) and silver/zinc oxide (Ag/ZnOVI) nanostructures using *V. indica (L.)* fruit extract. The nanostructures obtained were characterized to scrutinize their formation, crystal phase, size, shape, and surface properties via FT-IR, P-XRD, FE-SEM, EDS, and BET analysis. The *in-vitro* cytotoxicity of synthesized nanostructures was studied on the human triple-negative breast cancer (TNBC) cells. Besides, the stage of apoptosis was determined using AO-EB dual staining method. Furthermore, the DPPH free radical scavenging capacity, *in-vitro* anti-inflammatory, and *in-vitro* antidiabetic activity was performed. The results obtained have been discussed in detail.



Chapter 6a:

***Sauropus androgynus (L.)* phytochemical capped magnasium oxide nanoparticles: An investigation of substantial influence of zinc doping on their morphological, structural, and antimicrobial properties**

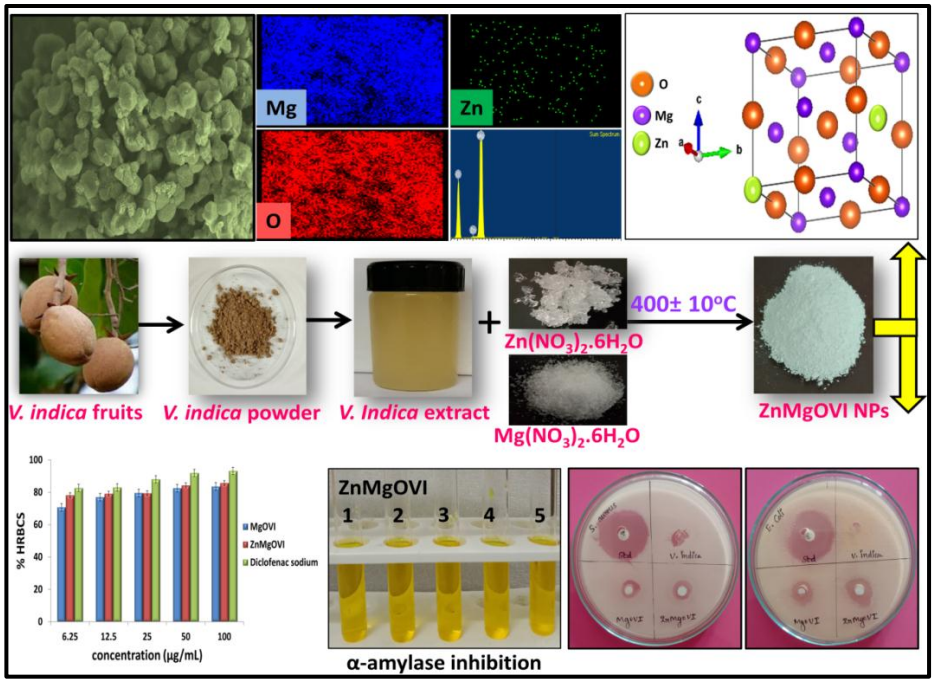
The framework of this chapter includes a study on the green synthesis of magnesium oxide (MgO) NPs. Pure (MgOSA) and zinc doped (ZnMgOSA) NPs were synthesized using an aqueous leaf extract of *S. androgynus (L.)*. The NPs synthesized were analyzed through FT-IR, P-XRD, FE-SEM, EDS, and BET techniques. The refinement of obtained P-XRD data through Rietveld refinement was performed to corroborate the changes occurred in the cell parameters after zinc doping. The NPs were scrutinized for their *in-vitro* antidiabetic activity using α-amylase inhibition assay and the antimicrobial activity against *Staphylococcus aureus*, *Bacillus subtulis*, *Pseudomonas aeroginosa*, *Pseudomonas syringae*, and *Escherichia coli*. Also their biocompatibility was evaluated using human red blood corpuscles. The details have been discussed.



Chapter 6b:

Insight into the impact of zinc doping on the structural, surface, and biological properties of magnesium oxide nanoparticles stabilized by *Vateria indica* (*L.*) fruit extract

This chapter delineates an attempt made to illustrate the effect of zinc doping on the green synthesized magnesium oxide (MgO) NPs. Pure (MgOVI) and zinc doped (ZnMgOVI) NPs were synthesized employing an aqueous fruit extract of *V. indica (L.)* as capping and reducing agent. The various analytical techniques viz. FT-IR, P-XRD, FE-SEM, EDS, and BET were used to characterize the synthesized NPs. Additionally; the refinement of obtained P-XRD data through Rietveld refinement was performed to corroborate the changes occurred in the cell parameters after zinc doping. The *in-vitro* antidiabetic activity was evaluated through α-amylase inhibition assay and the antibacterial activity study was conducted against *Staphylococcus aureus*, *Bacillus subtulis*, *Pseudomonas aeroginosa*, *Pseudomonas syringae*, and *Escherichia coli*. The results obtained have been discussed in detail.



Summary & Conclusion

The influence of stoichiometry of redox mixture on structural and the biological properties are compared. The conclusions derived from the studies are reported.

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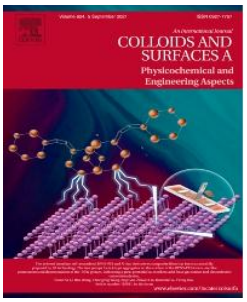
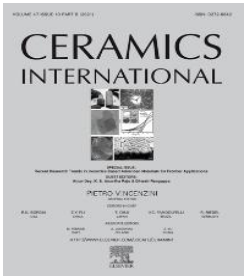
List of publications

[1] J.N. D'Souza, G.K. Nagaraja, A. Prabhu, K.M. Navada, S. Kouser, D.J. Manasa, Sauropus androgynus (L.) leaf phytochemical activated biocompatible zinc oxide nanoparticles: An antineoplastic agent against human triple negative breast cancer and a potent nanocatalyst for dye degradation, Appl. Surf. Sci. 552 (2021) 149429. DOI: 10.1016/j.apsusc.2021.149429. (ELSEVIER, Impact factor: 6.707)

[2] J. N. D'Souza, A. Prabhu, G. K. Nagaraja, K.M. Navada, S. Kouser, D. J. Manasa, Unravelling the human triple negative breast cancer suppressive activity of biocompatible zinc oxide nanostructures influenced by Vateria indica (L.) fruit phytochemicals, Mater. Sci. Eng. C. 122 (2021) 111887. DOI: 10.1016/j.msec.2021.111887. (ELSEVIER, Impact factor: 7.328)



[3] J. N. D'Souza, G.K. Nagaraja, K.M. Navada, S. Kouser, R. Nityasree, D.J. Manasa, An ensuing repercussion of solvent alteration on biological and photocatalytic efficacy of Emilia sonchifolia (L .) phytochemicals capped zinc oxide nanoparticles, Colloids Surfaces A Physicochem. Eng. Asp. 627 (2021) 127162. DOI:10.1016/j.colsurfa.2021.127162. (ELSEVIER, Impact factor: 4.539)



[4] J. N. D'Souza, G. K. Nagaraja, K.M. Navada, S. Kouser, D. J. Manasa, Insight into the impact of zinc doping on the structural, surface, and biological properties of magnasium oxide nanoparticles stabilized by *Vateria indica* (*L.*) fruit extract. Ceram. Int.(2021). DOI:10.1016/j.ceramint.2021.07.131. (ELSEVIER, Impact factor: 4.527)

Research papers under communication

[1] J. N. D'Souza, G. K. Nagaraja, K.M. Navada, S. Kouser, D. J. Manasa, *Sauropus androgynus (L.)* phytochemical capped magnasium oxide nanoparticles: An investigation of substantial influence of zinc doping on their morphological, structural, and antimicrobial properties.

[2] J.N. D'Souza, G.K. Nagaraja, A. Prabhu, K.M. Navada, S. Kouser, D.J. Manasa, Bio-fabrication of Ag and Ag/ZnO nanoparticles using *Sauropus androgynus (L.)* extract and evaluation of their cytotoxicity against MDA-MB468 Human Triple-negative breast cancer cells.

[3] J.N. D'Souza, G.K. Nagaraja, A. Prabhu, K.M. Navada, S. Kouser, D.J. Manasa, Antiproliferative activity of *Vateria indica (L.)* phytochemical influenced Ag/ZnO nanocomposite against Human Triple-negative breast cancer cells.

Papers presented in conferences

[1] Presented poster on 'Phyto-assisted synthesis and characterization of crystalline Zinc oxide nanoparticles' in the International conference on "Advanced Functional Materials for Energy, Environmental and Health Care (AFMEEHC), organized by Center for materials science and technology, University of Mysore, 18-20, March-2019.

[2] Presented poster on '*Vateria indica* fruit extract mediated synthesis of ZnO nanostructures: A potent nanocatalyst for cationic dye degradation and a free radical scavenger' in the International conference on "Advances in Chemical and Material Sciences", organized by Department of Chemistry, Mangalore University, 17-19th October-2019.

[3] Presented poster on 'Structural and optical properties of nano-structured Zinc Oxide materials synthesized by Solution Combustion method' in the National conference on "Novel Materials and Devices for Future Applications" (NMDFA), organized by St. Aloysius College, 18th February-2020

[4] Oral presentation on "Antiproliferative activity of *Vateria indica (L.)* phytochemical influenced Ag and Ag/ZnO nanostructures against human triple-negative breast cancer cells" in the international conference on "Emerging trends in Nanoscience and Nanotechnology (ICETNN), organized by Srinivas University , College of engineering and technology, 6-7th August-2021 (**Young Scientist Award**)

Prof. G.K. Nagaraja
Research Guide

Mrs. Josline Neetha D'Souza
Research Student